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Development of a Population Pharmacokinetic Model To Describe Azithromycin Whole-Blood and Plasma Concentrations over Time in Healthy Subjects

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Azithromycin (AZI), a broad-spectrum antibiotic, accumulates in polymorphonuclear cells and peripheral blood mononuclear cells. The distribution of AZI in proinflammatory cells may be important to the anti-inflammatory properties. Previous studies have described plasma AZI pharmacokinetics. The objective of this study was to describe the pharmacokinetics of AZI in whole blood (concentration in whole blood [C_b]) and plasma (concentration in plasma [C_p]) of healthy subjects. In this study, 12 subjects received AZI (500 mg once a day for 3 days). AZI C_b and C_p were quantified in serial samples collected up to 3 weeks after the last dose and analyzed using noncompartmental and compartmental methods. After the last dose, C_b was greater than C_p. Importantly, C_b, but not C_p, was quantifiable in all but one subject at 3 weeks. The blood area under the curve during a 24-h dosing interval (AUC_{24}) was ~2-fold greater than the plasma AUC_{24}, but simulations suggested that C_b was not at steady state by day 3. Upon exploration of numerous models, an empirical 3-compartment model adequately described C_p and C_b, but C_p was somewhat underestimated. Intercompartmental clearance (CL; likely representing cells) was lower than apparent oral CL (18 versus 118 liters/h). Plasma, peripheral, and cell compartmental volumes were 439 liters, 2,980 liters, and 3,084 liters, respectively. Interindividual variability in CL was low (26.2%), while the volume of distribution variability was high (107%). This is the first report to describe AZI C_b in healthy subjects, the distribution parameters between C_p and C_b, and AZI retention in blood for up to 3 weeks following 3 daily doses. The model can be used to predict C_b from C_p for AZI under various dosing regimens. (This study has been registered at ClinicalTrials.gov under registration no. NCT01026064.)

Azithromycin (AZI) is a 15-membered lactone-ring azalide structurally related to the macrolide family of antibiotics. AZI is approved for use worldwide as a broad-spectrum antibiotic to treat a variety of community-acquired infections. It acts by binding to the 50S ribosomal subunit of susceptible organisms and interfering with protein synthesis (1, 2). In addition, AZI and other macrolides are investigational products with anti-inflammatory/immunomodulatory actions that may be beneficial for those suffering from chronic pulmonary inflammatory syndromes, such as cystic fibrosis (CF), bronchiolitis obliterans syndrome (BOS), chronic obstructive pulmonary disease (COPD), asthma, and bronchiectasis (3–5).

The recommended dose of AZI for the treatment of acute respiratory tract infections is 500 mg (10 mg/kg of body weight in children) once daily for 3 days or 500 mg (10 mg/kg) on day 1, followed by 250 mg (5 mg/kg) once daily for 4 days (6). The optimal dose and dosing regimen of AZI for the treatment of chronic lung inflammation, where chronic dosing is required for clinical benefit, are unknown.

Various empirically selected dosing regimens were studied in patients with chronic pulmonary inflammatory diseases. For example, long-term, once-daily dosing was tested in CF patients at dose levels of 250 or 500 mg (or the pediatric equivalent of 15 mg/kg, which corresponds to an adult dose of 750 mg) (7–13). Once-daily dosing of 250 or 500 mg was also tested in patients with COPD or asthma, respectively (14, 15). Dosing three times weekly was evaluated in patients with BOS (250 mg thrice weekly with or without a loading dose of 1,250 mg in the first week), bronchiectasis (250 mg thrice weekly), CF (500 mg thrice weekly), COPD (500 mg once daily over 3 days each week), and asthma (10 mg/kg once daily over 3 days each week) (16–26). Twice-weekly dosing of 250 mg was assessed in patients with asthma or COPD with or without a loading dose, and a dose of 500 mg twice weekly was tested in patients with bronchiectasis (27–29). Finally, once-weekly dosing was evaluated in patients with asthma (600 mg once weekly with a loading dose of 1,800 mg or 1,000 mg once weekly) and CF (1,000 to 1,250 mg or the pediatric equivalent) (10, 12, 30–33). Other reported regimens included 500 mg daily for 6 days, followed by 250 mg daily for 6 days, and weekly dosing of 250 mg daily for 3 days thereafter (in patients with bronchiectasis) and 500 mg daily over 3 days every 3 weeks (in patients with COPD) (34, 35).

Despite such variety in dosing regimens and total weekly doses, which ranged from 500 to 5,250 mg (adult equivalent of 15 mg/kg daily for 7 days), beneficial effects of AZI were detected across the trials and indications studied. Furthermore, a couple of studies that compared different AZI regimens in CF patients did not show

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a major difference in efficacy or safety between a low (5 mg/kg) and a high (15 mg/kg) daily dose of AZI or between 250 mg daily and 1,200 mg weekly (9, 10). An improved understanding of the systemic exposures in whole blood achieved after long-term treatment with various dosing regimens of AZI, along with clarification of its anti-inflammatory mode of action, would aid the interpretation of the observed efficacy results and enable a rational selection of doses and dosing schedules for clinical trials of AZI in chronic inflammatory diseases.

In contrast to other macrolide agents, AZI distributes and accumulates extensively in a number of cells, including peripheral blood mononuclear cells (PBMCs), polymorphonuclear leukocytes (PMNs), and fibroblasts (36–39). The exceptionally high accumulation of AZI in tissues is consistent with the ubiquitous nature of the fibroblasts, which are cells found in virtually all tissues (36, 40, 41). Consistent with the dibasic character of AZI, cell fractionation studies showed that most of the cellular and tissue AZI is stored in lysosomes (39, 42). While AZI accumulates extensively in white blood cells, several studies showed little or no accumulation of AZI in erythrocytes (38, 43).

The pharmacokinetic profile of AZI is consistent with the rapid and extensive uptake of this compound from the circulation into intracellular compartments, followed by slow release. These distributional attributes of AZI lead to low serum concentrations, widespread tissue distribution, a very long serum terminal half-life of 78.6 h, and a large volume of distribution (V) (38, 41). AZI binds to plasma proteins in a nonlinear fashion; the bound fraction decreases from 51% at low concentrations in serum to 7% at higher concentrations in serum (41).

Extensive accumulation of AZI in proinflammatory cells, in combination with bactericidal activity, has been proposed to contribute to the eradication of the phagocytized organisms and to be relevant to the therapeutic efficacy of AZI against certain infections (43, 44). In addition, AZI reduces inflammatory cell migration by direct effects on neutrophils (45) and modifies monocyte activation and differentiation (46, 47). Therefore, AZI accumulation in proinflammatory cells may be important to the anti-inflammatory properties of AZI.

Studies published to date have characterized the pharmacokinetic parameters of AZI in plasma and its accumulation in various types of isolated white blood cells in either healthy or CF patients (48–51). However, no pharmacokinetic investigation has been carried out to simultaneously address the distribution of AZI between plasma and whole blood in healthy subjects. The aim of this study was to develop a population pharmacokinetic model to characterize the concentration-time profile of AZI in plasma and whole blood following three 500-mg doses in healthy male volunteers and to simulate the pharmacokinetic profiles of AZI upon long-term administration.

MATERIALS AND METHODS

Study design and subjects. Data were obtained from a study designed to address the utility of the cantharidin-induced skin blister assay to evaluate the anti-inflammatory effects of drugs in healthy volunteers. The study was designed as a randomized, double-blind, placebo-controlled, parallel group trial. Twenty-five healthy male volunteers were randomized in a 1:1 ratio to receive AZI (500 mg once daily over 3 days) or placebo. Eligibility criteria included age between 18 and 65 years, body weight above 50 kg, and body mass index between 18.5 and 30 kg/m^2. Subjects with a history of HIV infection, hepatitis B or C, or sensitivity to AZI or macrolide/ketolide antibiotics were excluded from the study. The clinical protocol was approved by the Essex 2 Research Ethics Committee (09/H0302/50), Cambridge, United Kingdom, and informed consent was obtained from each subject prior to entering the study. This study was conducted in accordance with good clinical practice (GCP) and all applicable regulatory requirements and the guiding principles of the Declaration of Helsinki. The ClinicalTrials.gov identifier of this study is NCT01026064.

Drug administration and sample collection. Two capsules of 250 mg AZI (Zithromax; Pfizer Ltd.) or matched placebo (Pharmaceutical Development, GSK, Verona, Italy) were administered once daily for three consecutive days with ~240 ml of water at 1 h before or 2 h after a meal. Blood samples of 1 ml and 4 ml were collected before the last dose of study medication and at 1, 2, 3, 4, 8, 24, 48, and 504 h (3 weeks) postdose for the measurement of blood and plasma AZI concentrations, respectively.

Bioanalytical methods. AZI analysis was performed under the management of Worldwide Bioanalysis, DMPK, Verona, Italy, GlaxoSmithKline. AZI concentrations were measured in hemolyzed blood (after 1:1 dilution with distilled water) and in plasma samples by high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) using a validated analytical method. Samples were prepared by protein precipitation. The lower limit of quantitation (LLOQ) of the assay was 2 ng/ml for plasma and 4 ng/ml for blood. The within-run precision was ≤7.1% for both the plasma and blood assays. The between-run precision was ≤9.5% and ≤3.5% for plasma and blood, respectively. The assay accuracy was between 14.4% and 10.2% for plasma and between −15% and 2.7% for blood.

Data analysis. The following noncompartmental pharmacokinetic parameters were calculated for day 3 data using the Phoenix WinNonLin (version 6.2) program (Pharsight Corporation): maximum plasma concentration (C_{max}), the area under the curve during a 24-h dosing interval (AUC_{24}), and half-life. Compartmental population pharmacokinetic models were developed using the first-order conditional estimation method with interaction in the Phoenix NLME (version 1.1) program (Pharsight Corporation). Model selection was based on the following criteria: (i) successful minimization; (ii) visual inspection of goodness-of-fit plots; (iii) a difference in objective function value ($-2 \cdot \log$ likelihood) of more than 3.84, corresponding to a significance level (P value) of <0.05, on the basis of the assumption of the objective functions for two nested models differing by 1 degree of freedom; (iv) relative standard errors of the parameter estimates; and (v) successful completion of the covariance step. If the models being compared were not nested, the model selection was based on goodness-of-fit plots and the Akaike information criterion. Due to the narrow range of weights and the small number of subjects, no subject characteristic could be included as a covariate in the model.

Pharmacokinetic model development. Model development was performed in several steps. First, an exploratory analysis was undertaken to investigate the basic structure of the plasma and blood concentration-time profiles (alone and combined), including absorption (e.g., lag time, transit compartments), distribution, and elimination components. Then numerous integrated 3- and 4-compartment models in a catenary or mammillary structural arrangement were explored to simultaneously model the plasma and whole-blood concentration-time data. A brief, nonexhaustive synopsis of the numerous compartmental and semiphysiological models explored is presented in Fig. S1 in the supplemental material.

The final model chosen on the basis of goodness-of-fit criteria was a 3-compartment mammillary model with first-order absorption, lag time, and first-order elimination from the central compartment (see model 3 in Fig. S1 in the supplemental material), in which the relationship between plasma and blood concentrations over time was described using an empirical equation:

$$C_b = M \cdot C_p + N \cdot C_{bc} \quad (1)$$

where C_b, C_{bc}, and C_p are the whole blood, blood cell, and total plasma concentrations of AZI, respectively, and M and N are empirical, model-estimated parameters (empirical coefficients to blood cell concentration and plasma concentration, respectively). The differential equations of the 3-compartment model and the approach used to determine the appropriate relationship between blood and plasma concentrations are presented

TABLE 1 Pharmacokinetic parameters determined by noncompartmental analysis[a]

Matrix	C_{max} (μg/liter)	T_{max} (h)	AUC_{24} (μg · h/liter) on day 3	$t_{1/2}$ (h)	V_z/F (liters)	CL/*F* (liter/h)
Blood	772 (39.0)	3.00 (1.95–4.00)	7,893 (30.4)	117 (33.1)	10,700 (33.6)	63.3 (30.4)
Plasma	500 (44.4)	2.02 (1.95–3.95)	3,468 (27.5)	27.2 (20.6)[b]	5,670 (33.1)	144 (27.5)

[a] Data represent geometric mean values (geometric coefficient of variation [in percent]) for all parameters except the time to the maximum concentration in plasma, which is presented as the median (range). C_{max}, maximum concentration in plasma; T_{max}, time to maximum concentration in plasma; AUC_{24}, area under the curve during a 24-h dosing interval; $t_{1/2}$, half-life; V_z/F, volume of distribution based on the terminal phase; CL/*F*, clearance.

[b] Plasma concentrations were quantifiable up to 48 h in all subjects. Therefore, due to the limited number of data points available, the half-life in plasma should be interpreted with caution.

in the section "Pharmacokinetic model development" in the supplemental material.

Pharmacostatistical model. An exponential variance model was used to describe the interindividual variability:

$$P_i = P_{pop} \cdot \exp(\eta_i) \quad (2)$$

where P_i is the individual parameter estimate in the *i*th individual, P_{pop} is an estimate of the population mean of parameter *P*, and η_i is the deviation from the population mean for the *i*th individual under the assumption that η's are normally distributed with mean 0 and variance ω^2. Random effects were initially modeled on clearance (CL) and the volume of the central compartment. The requirement for additional random effects was investigated by sequentially adding or removing them from the model. When multiple random effects were included, both diagonal and full block covariance matrices were explored.

For the residual variability, proportional error models were used for both blood and plasma:

$$Y_{ij} = C_{ij} \cdot (1 + \varepsilon_{ij}) \quad (3)$$

where Y_{ij} is the observed blood or plasma concentration in the *i*th individual at the *j*th time point, C_{ij} is the predicted concentration, and ε_{ij} is the proportional residual error term under the assumption that $\varepsilon \sim N(0, \sigma^2)$. In addition, additive residual models were also explored but were rejected on the basis of goodness-of-fit plots. Separate residual terms were used for the blood and plasma measurements.

Model qualification. The final model was evaluated using the nonparametric bootstrap and visual predictive check options in Phoenix NLME (version 1.1). The precision of the parameter estimates was assessed using a bootstrap analysis with 1,000 data sets, randomly sampled from the original data set, with replacement. The final pharmacokinetic model was fit to each of the data sets; the mean, standard error (SE), and 95% confidence intervals (CIs) of the model parameters were calculated and compared with the final parameter estimates and SEs generated in Phoenix NLME. A visual predictive check (VPC) was also performed to evaluate the predictive properties of the model (n = 1,000 data sets).

Treatment of plasma concentrations BLQ at 3 weeks. Because concentrations in plasma samples, but not blood samples, were below the limit of quantification (BQL) at 3 weeks postdosing, initial analysis treated these BQL values as missing, which led to a model in which there was no difference in the accumulation of AZI in plasma or blood upon repeated dosing (an unexpected finding). Further assumptions (e.g., setting the BLQ concentrations to 0 or to one-half the LLOQ level) were not successful. The method of treating the reported BLQ concentrations as true values weighted by the increased variability associated with the LLOQ concentrations was not examined because the requisite data were not available. Furthermore, it was not possible to use the subject's terminal elimination half-life in plasma to estimate plasma concentrations at 3 weeks because the small duration of sample collection with quantifiable concentrations was too short (e.g., 48 h, <2 times the half-life). Therefore, the plasma BLQ concentrations at 3 weeks were extrapolated from the last measurable plasma concentration using a literature-reported half-life of 78.6 h (51, 52), which allowed different levels of accumulation between plasma and blood after long-term administration.

RESULTS

Subjects and exploratory analysis. The 12 male subjects who received AZI had a median age of 29 years (range, 19 to 47 years), a median height of 174.5 cm (range, 164 to 180 cm), and a median weight of 73 kg (range, 61.1 to 87.9 kg). Noncompartmental analysis showed that AUC_{24} was more than 2-fold higher in blood than plasma (Table 1). The mean (Fig. 1) as well as individual (data not shown) blood AZI concentrations were greater than the mean plasma concentrations over the entire sampling interval. These profiles are indicative of an extensive uptake of the drug from plasma into the blood cell compartment, consistent with previous reports of AZI accumulation in white blood cells.

Population pharmacokinetic analysis. The disposition of AZI in whole blood and plasma was adequately described by a 3-compartment model. The model was parameterized with a first-order absorption rate constant (k_a); absorption lag time (T_{lag}); apparent volume of distribution of the central compartment, assumed to be plasma (V_p/F); apparent volume of distribution of one peripheral compartment, assumed to be the blood cells (V_{bc}/F); apparent volume of distribution of the second peripheral compartment, assumed to be the fibroblasts in tissues (V_t/F); two apparent distributional clearances between the central (blood cell) and peripheral (tissue) compartments (CL_{bc}/F and CL_t/F, respectively); and an apparent elimination clearance (CL/*F*).

Assuming that the central compartment represents plasma and that one of the peripheral compartments represents the blood cells, the concentration in blood can be expressed as a function of the concentration in plasma, the concentration in blood cells, and the hematocrit (see "Pharmacokinetic model development" and equation 8 in the supplemental material). However, this relationship did not result in an acceptable model fit. This was probably

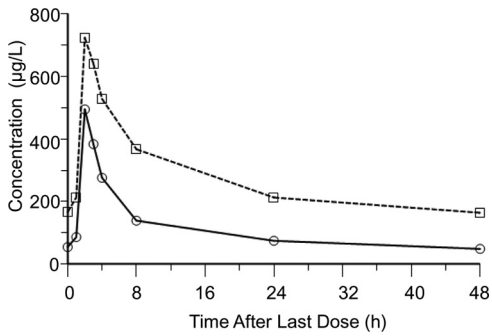

FIG 1 AZI blood (□) and plasma (○) concentration-time profiles. Data represent the mean concentration-time profiles in blood and plasma for up to 48 h following the last of the three (500-mg) doses of AZI given once daily.

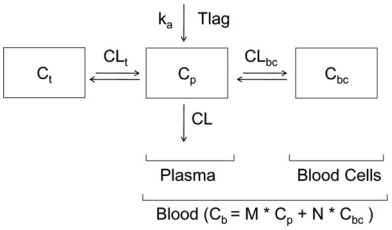

FIG 2 Diagram of the 3-compartment structural model for AZI concentrations in blood and plasma. k_a, absorption rate constant; T_{lag}, lag time; C_p, plasma concentration; C_t, concentration in peripheral compartment, assumed to be tissue; C_{bc}, concentration in peripheral compartment, assumed to be blood cells; CL_t, intercompartmental clearance to tissue compartment; CL_{bc}, intercompartmental clearance to blood cell compartment; CL, elimination clearance; *M*, empirical coefficient to blood cell concentration; *N*, empirical coefficient to plasma concentration.

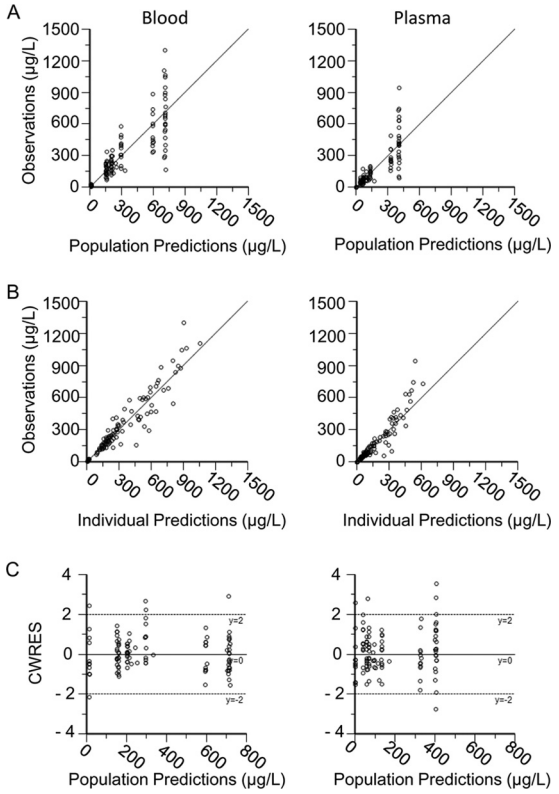

FIG 3 Goodness-of-fit graphics for blood (left) and plasma (right). Graphs depict the observed concentrations versus the population predictions (A), the observed concentrations versus the individual predictions (B), and the CWRES versus the population predictions (C). The line of identity is included in panels A and B.

due in part to insufficient data to accurately describe the intracellular sequestration of AZI in lysosomes, the kinetics of release from the cellular compartment, as well as the nonlinear kinetics of AZI binding to plasma proteins. In addition, the hematocrit value is driven by red blood cells, which do not accumulate AZI. To compensate for this lack of information, a modified, empirical equation was used to describe the mathematical relationship between the concentrations in plasma and whole blood (equation 1).

The blood concentration in one subject and all the plasma concentrations at the 504-h time point (3 weeks) were below the LLOQ (BLQ). Ignoring the BLQ values at 504 h postdose resulted in a model which suggested that plasma concentrations remained elevated over 3 weeks; this was inconsistent with the observed data. Therefore, the missing plasma concentrations in each individual were calculated assuming exponential decay in the terminal elimination phase and using the last measurable plasma concentration and a previously published half-life of 78.6 h (51, 52) (see "Treatment of plasma concentrations BLQ at 3 weeks" in Materials and Methods).

The final model structure is shown in Fig. 2. Goodness-of-fit plots for observed versus population predicted, observed versus individual predicted, and conditional weighted residuals (CWRES) versus population predicted concentrations are presented in Fig. 3. The observed versus population predicted plots show a uniform distribution of the data points across the line of unity. The observed versus individual predicted concentration plots suggest a relatively small underprediction of the higher (peak) concentrations in both plasma and blood. CWRES versus population predicted concentrations showed little to no bias. Individual predictions of blood concentration-time and plasma concentration-time data after the last dose are shown in Fig. S2 and S3 in the supplemental material, respectively. Consistent with the observed versus individual predicted concentration plots, these graphs also show a slight underprediction of the peak concentrations in both the plasma and blood of some subjects.

Following a mean lag time of 0.95 h, absorption was rapid (k_a = 0.604 h^{-1}). The two peripheral compartments exhibited very large volumes of distribution, both of which were significantly larger than the volume of distribution in the central compartment, consistent with the known extensive distribution of AZI in peripheral tissues and cells. Thus, V_{bc}/F was 3,084 liters and V_t/F was 2,980 liters, while V_p/F was 440 liters. The distributional clearance into the blood cell compartment was significantly smaller than the distributional clearance into the second peripheral compartment (17.8 versus 213 liters/h). The uncertainty, expressed as the percent relative standard error (RSE) for the fixed-effects parameters, was low to moderate, ranging from 6.8 to 43.7%. The magnitude of the interindividual variability (percent coefficient of variation [CV]) was low in CL_t/F (8.2%), moderate in the elimination CL/*F* and absorption rate constant (30.3% and 26.2%, respectively), and high in V_p/F (107.3%). Interindividual variability could not be estimated for the intercompartmental distributional clearances or for V_{bc}/F. Proportional residual error (percent CV) was estimated to be 24.1% in plasma and 23.2% in blood. Shrinkage was calculated for interindividual and residual variability parameters, and it was below 20% in all cases (53).

Model qualification. The diagnostic plots (Fig. 3) indicated that the model adequately described the data. The results of the bootstraps, expressed as the median (95% CI) parameter estimates, are presented in Table 2. The median from the parameter bootstrap was within 10% for the typical values of CL/*F*, V_{bc}/F, CL_{bc}/F, V_t/F, T_{lag}, *M*, and *N* and within 26% for the typical values of V_p/F and CL_t/F. For k_a, the median from the parameter bootstrap was within 44% of the typical value. Visual predictive check plots for plasma and whole blood show that most of the concentrations observed in both plasma and blood were within the predicted 5% and 95% quantiles (Fig. 4).

Simulation of plasma and blood concentrations after long-term administration. The final model was used to predict the

TABLE 2 Parameter estimates by bootstrap analysis

Model parameter	Symbol (units)	Bootstrap estimate[a]			% CV
			95% CI		
		Median	Lower	Upper	
Absorption rate constant	k_a (h^{-1})	1.06	0.485	5.820	
Lag time	T_{lag} (h)	1.25	0.93	1.94	
Plasma vol of distribution	V_p/F (liters)	452	40	1,036	
Blood cell vol of distribution	V_{bc}/F (liters)	3,001	2,360	3,871	
Tissue compartment vol of distribution	V_t/F (liters)	2,785	2,227	3,593	
Elimination clearance	CL/F (liters/h)	115	100	134	
Intercompartmental clearance to blood cell compartment	CL_{bc}/F (liters/h)	16.1	11.5	25.2	
Intercompartmental clearance to tissue compartment	CL_t/F (liters/h)	289	167	554	
Blood cell concn coefficient in equation 1	M	1.64	1.53	1.74	
Plasma concn coefficient in equation 1	N	2.20	1.87	2.63	
IIV on[b]:					
k_a	$\omega^2_{k_a}$				58.0
CL/F	$\omega^2_{CL/F}$				24.9
Plasma V	$\omega^2_{V_p/F}$				100
CL_t/F	$\omega^2_{CL_t/F}$				29.5
Residual error					
Plasma	σ^2_p				24.6
Blood	σ^2_b				20.8

[a] Bootstraps with 1,000 data sets were performed.
[b] IIV, interindividual variability.

concentration-time profiles of AZI in plasma and blood after prolonged administration of 500 mg AZI once daily for 30 days. The simulation results are displayed in Fig. 5 and show that AZI reaches steady-state concentrations in plasma at 5 days, while AZI continues to accumulate in whole blood even after 30 days of administration. Furthermore, these simulations predict a mean blood/plasma accumulation ratio of ~4-fold following long-term once-daily administration of 500 mg AZI. This ratio is in line with the 5-fold accumulation ratio calculated on the basis of blood and plasma concentration data from cystic fibrosis patients following long-term daily administration of 500 mg AZI (49).

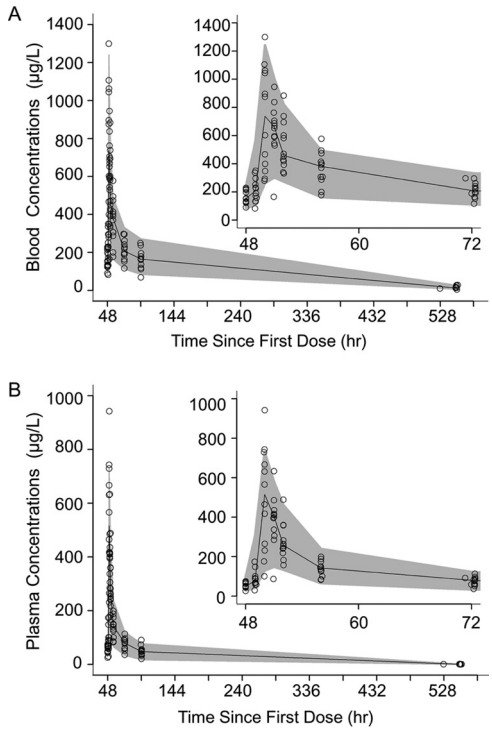

FIG 4 Visual predictive check (VPC) in blood (A) and plasma (B). Open circles, observed data points; solid line, predicted 50th percentile; shaded area, area between the predicted 5th and 95th quantiles. Ideally, 90% of the observations should fall inside the 90% prediction interval.

DISCUSSION

Given the pharmacodynamics and unique plasma and blood cell pharmacokinetics of AZI, the majority of studies to date have focused on AZI accumulation in PMNs or PBMCs and on plasma distribution. Freeman et al. (44) demonstrated that AZI accumulates in PMNs and that PMNs serve as a unique system for delivery of the drug to the site of infection. Olsen et al. (54) demonstrated that AZI accumulates extensively in PBMCs. Ballow and Amsden provided additional evidence for AZI accumulation in PMNs and studied the pharmacokinetics of the AZI distribution between plasma and PMNs using a compartmental modeling approach

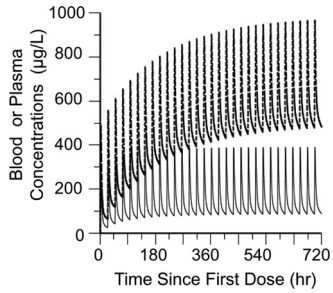

FIG 5 Predictions of AZI accumulation in blood and plasma after long-term (30 days) administration of 500 mg daily. Bold, dashed lines, predicted blood concentration-time profile; thin, solid lines, predicted plasma concentration-time profile.

(38). Due to the demonstrated importance of AZI accumulation in both PMNs and PBMCs, as well as the fact that AZI may distribute transiently into erythrocytes (55), we aimed to develop an all-inclusive pharmacokinetic model that described the relationship between plasma and whole-blood AZI concentrations following the last dose of a 500-mg-once-daily regimen for 3 consecutive days.

A nonlinear mixed-effects population modeling approach was employed. A three-compartment model with first-order absorption, lag time, and first-order elimination best described the blood and plasma disposition of AZI after oral administration of three 500-mg daily doses. Blood concentrations were described using an empirical approach based on the assumption that plasma concentrations were sampled from the central compartment and that one of the peripheral compartments represented the blood cells.

Previously, Ballow and Amsden studied the compartmental distribution of AZI based on the comodeling of plasma and urine data (51). The final model included two peripheral compartments differing in rates of equilibration: one fast and one slow (CL_{df}/F = 61.3 liters/h and CL_{ds}/F = 253 liters/h, respectively), where CL_{df}/F and CL_{ds}/F are the distributional clearances of the fast and slow equilibrating peripheral compartments, respectively. In addition, the two peripheral compartments had very large volumes of distribution of 4,304 liters and 3,707 liters, respectively, and a reported elimination CL of 153 liters/h. The population pharmacokinetic model presented here resulted in similar (although not identical) fixed parameter values.

A number of limitations of this model could be identified. First, the model-estimated interindividual variability in V_p/F was high (107.3%). This might be explained, in part, by the absence of data to properly describe the nonlinear binding of AZI to plasma proteins observed over the range of therapeutic concentrations (41). Second, bootstrap analysis showed a high degree of uncertainty in the k_a parameter estimate. Third, plots of the predicted versus measured concentrations showed that the peak concentrations were underpredicted in both plasma and blood. This underprediction was not improved by adding transit compartments, deleting the lag time, or changing intersubject variability on these parameters. Fourth, using the literature-published plasma half-life of 78.6 h to extrapolate the plasma concentration at 3 weeks limits the description of interindividual variability in CL and V. Lastly, after exploring over 350 models in total, an empirically derived equation was employed to describe the relationship between blood and plasma concentrations (equation 1). The fact that we were not able to use a physiological relationship may be due to the lack of information regarding the nonlinear binding of AZI to plasma proteins and to the kinetics of intracellular drug uptake and sequestration in lysosomes. Even with these limitations, the model was well described and able to predict concentrations after daily dosing of AZI in CF patients.

A phase III study (22) showed that AZI, although investigational, is effective in subjects with CF who are chronically infected with *Pseudomonas aeruginosa*. In addition, long-term therapy with 250-mg daily doses of AZI for 1 year was shown to decrease the frequency of exacerbations in COPD patients (14), and long-term treatment with 250 mg thrice weekly was effective in prevention and treatment of BOS in lung transplant patients (25). The promising clinical data on AZI efficacy in patients with bronchiectasis or severe persistent asthma need to be confirmed in randomized, placebo-controlled studies (16, 27, 33, 34).

While diverse dosing regimens of AZI were tested in patients with chronic inflammation of the lower airways, limited data about the systemic exposure achieved in these patients exist. Wilms et al. reported that after at least 35 days of once-daily treatment with 500 mg AZI in CF patients, the C_{max} in plasma was 0.67 μg/ml and the AUC_{24} was 5.3 μg · h/ml (49). The corresponding values in blood were 2.01 μg/ml and 27.8 μg · h/ml; i.e., they were 3- and 5-fold higher than those in plasma, respectively. Elimination half-lives were 102 h in plasma and 178 h in blood (49). A subsequent study in CF patients treated with 1,000 mg AZI once weekly for 3 months revealed that AUC_{168} was 3.5 times higher in blood than in plasma (12). Thus, the ability to predict the accumulation of AZI in patients after long-term administration is clinically relevant.

To determine AZI concentration-time profiles after long-term administration, the plasma and blood concentrations were simulated using a conservative, deterministic simulation based on the current data set, for which the individual interindividual variability was determined. The simulation results presented here showed a 4-fold accumulation in blood compared to that in plasma, consistent with the observations in CF patients. This suggests that both plasma and blood concentrations of AZI should be considered in establishing a pharmacokinetic/pharmacodynamic relationship in nonclinical and clinical studies. Considering that AZI accumulates in white blood cells and modulates their function, it is likely that whole-blood concentrations are more predictive of efficacy than plasma concentrations (49). Furthermore, in spite of the limitations discussed above, the fact that the blood/plasma accumulation predicted by the simulation presented is in line with the blood/plasma accumulation observed in CF patients provides an external validation of our model and suggests that the model is acceptable for future use in predicting the blood exposures following long-term administration of AZI in different chronic inflammatory diseases.

In conclusion, the presented population pharmacokinetic model describing AZI concentrations in plasma and blood after repeated oral dosing can be utilized to predict the plasma and blood concentrations under various AZI dosing regimens. These data may be used to outline the clinically effective exposure in investigational studies in patients with CF, COPD, or BOS and select the most promising treatment schedules for patients with other chronic inflammatory lung diseases. Also, the model may be used as a starting point for prediction of long-term exposure in humans of novel compounds which exhibit an AZI-like pharmacokinetic disposition.

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